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The evaluation of the microbial community of lower respiratory tracts microbiota in tuberculosis patients and healthy individuals using Metagenomics

Kiarash Ghazvini (MD)[1,2], Masoud Youssefi (MD)[1,2], Masoud Keikha (MD)[1,2]*

[1]*Department of Microbiology and Virology, Faculty of Medicine, Mashhad University of Medical Sciences, Mashhad, Iran.*
[2]*Antimicrobial Resistance Research Center, Mashhad University of Medical Sciences, Mashhad, Iran.*

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ABSTRACT

Introduction: Tuberculosis is remained as global challenge which is considered as the top cause of human death in worldwide. The presence of lower respiratory tracts microflora can modulate immune response and play important role in susceptibility to TB. The aim of study was comparison of microbial diversity in lower respiratory tracts microflora of pulmonary tuberculosis patinas and healthy individuals
Methods: In this study, the raw sequences of SRR493275 and SRR493275 were retrieved from European Bioinformatics Institute online database. Then, the raw sequences were filtered by their quality (adapter contamination, low quality as well as low complexity reads) and taxonomic analyzed by online websites including Galaxy/CRS4 and KAIJU online servers. The statistical analysis was conducted to evaluate the presence of significant microbial diversity between two groups.
Results: We found that microbial taxa were similar between TB and normal except Tenericutes which supplemented in microflora of pulmonary tuberculosis cases. Moreover, the abundance of bacterial genera is significantly divers between TB and healthy groups.
Conclusion: There is significant diversities in the lower respiratory tracts microflora of TB and controls. Increasing the abundance of anaerobic genera in TB patients may be suppressed immune response and essential for susceptibility to active pulmonary tuberculosis.

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Introduction

Although it's a century since Robert Koch introduced the *Mycobacterium tuberculosis* as a causative agent of tuberculosis (TB), this remains one of the leading causes of death throughout the globe (1, 2). According to WHO reports in 2020, about 10 million individuals have become infected with tuberculosis, and 1.5 million have died in 2019 (3). In recent years, tuberculosis eradication seems to be impossible due to the inefficacy of the BCG vaccine in adults, the proliferation of patients with immunodeficiency, HIV pandemics, and drug-resistance TB (4-7).

Pulmonary tuberculosis follows a chronic infection characterized by the formation of granuloma lesion; also, the formation of granuloma is caused by the equilibrium between the host immune system and *M. tuberculosis*. In the case of granuloma formation, there is an equilibrium between Th1

***Corresponding author:** Masoud Keikha.
Department of Microbiology and Virology, Faculty of Medicine, Mashhad University of Medical Sciences, Mashhad, Iran.
***E-mail:** masoud.keykha90@gmail.com*
***Tel:** +989386836425*

and Th2 responses, and any factor that disrupts this equilibrium leads to the induction of caseous necrosis and active TB (8, 9). Numerous factors such as pathogenicity and virulence of *M. tuberculosis*, epigenetic events, genome polymorphism, immune system, and microbial flora can play an important role in immune-pathogenesis of tuberculosis (10-12). Initially, it was supposed that the lower respiratory tracts are sterile and lack microbial flora, but recent studies have shown that these tracts are not sterile and have microbiota similar to upper respiratory tracts (13). Microbiota can affect the responses of the immune system and may change in various diseases, indicating the effect of microflora on the modulation of immune response and human hemostasis. However, limited studies have been performed on the role of the human microbiome and tuberculosis (14,15).

The present study aimed at evaluating of the microbial community of and determining the population structure of lower respiratory tracts microbiota in patients with pulmonary tuberculosis and healthy subjects.

Methods

In this study, potential relevant assays were selected from studies deposited in the Category of Genomes & Metagenomes available in the European Bioinformatics Institute database (at: https://www.ebi.ac.uk/). In this process, candidate studies were collected using the keywords "*Mycobacterium tuberculosis*", "Tuberculosis", "Microbiota" and "16S rRNA" up to May 2020. The inclusion criteria were: 1. Metagenomics studies using hypervariable areas of the 16S rRNA gene, 2. The sample should be bronchoalveolar lavage fluid (BAL), 3. Pulmonary tuberculosis in patients should be confirmed using sputum staining and radiological findings methods, and 4. The quality of sequencing data of studies should be at a satisfactory level. Studies that did not meet such criteria were excluded. Also, another study on microflora in a healthy population (as a control) was selected for statistical analysis and comparison of lower respiratory tract microbiota in tuberculosis patients and healthy individuals.

Raw sequencing reads were retrieved, de-multiplexed, filtered by their quality (adapter contamination, low quality as well as low complexity reads) and taxonomic analyzed by online websites including Galaxy/CRS4 (at https://orione.crs4.it/) and KAIJU (from http://kaiju.binf.ku.dk/server). Finally, to indicate the differences of the lower respiratory tract microflora between tuberculosis patients and healthy individuals, comprehensive statistical analysis was carried out in Microsoft Excel version 2016.

Results

We found that two studies with the accession number of SRR617950 were analyzed as case subjects (tuberculosis patients) and SRR493275 as healthy subjects due to matching our criteria. Particularly, in both groups evaluated in the present study, lower respiratory tract microbiota was taken from BAL samples.

A total of 278,632 raw 16S rRNA reads were obtained from the case (253,386) and control (25,246) subjects. After filtering the process on low-quality reads, about 9% of raw sequence reads were deleted. And high-quality raw read sequences with an average read length of about 200 bp were analyzed taxonomically. The major bacterial phyla in the case group (TB patients) included *Firmicutes, Proteobacteria, Actinobacteria, Fusobacteria, Bcteroides* and *Tenericutes*, respectively, while in the control group (healthy individuals) the distribution of bacterial phyla included *Firmicutes, Proteobacteria, Clostridia, Actinobacteria, Fusobacteria*, and *Bcteroides*, respectively. According to these results, it was found that the microflora in the lower respiratory tract of TB patients is different from that of healthy individuals. We observed significant variations in the distribution of *Actinobacteria, Firmicutes*, and *Proteobacteria* among tuberculosis patients and the healthy group, so that the frequency of bacterial phyla including *Proteobacteria* and *Firmicutes* varied significantly among the groups of tuberculosis patients and healthy individuals. However, microbial diversity was almost similar in the two groups. Also replacement of *Tenericutes* in the lower respiratory tract of tuberculosis patients was observed, but this phylum was not observed in the control community. However, the size of the evaluated sample was limited in the present metagenomics study and due to dissimilarity in the public structure we need to conduct further studies with larger sample size.

After identification to bacterial genera level, we found that the most dominant bacterial genera in tuberculosis patients included *Streptococcus, Propionibacterium, Mycobacteria, Staphylococcus, Lactobacillus, Neisseria, Ruminococcus, Fusobacterium, Thiomonas*, and *Leptotrichia*, while in the healthy group, the most dominant bacterial genera included *Lactobacillus, Streptococcus, Haemophilus, Burkholderia, Bacillus, Propionibacterium, Corynebacterium, Bacteroides, Vibrio*, and *Spirochaetae.* Also, in these groups, *Lactobacilus* and *Spirochaetae* constituted the highest and lowest microbiota populations in the lower respiratory tract, respectively.

Generally, microbial diversity was similar in both TB and control groups. However, the frequency of bacterial genera was different in the two groups,

so, based on the statistical analysis, the bacterial genus such as *Mycobacteria, Neisseria, Leptotrichia, Enetrobacteriace, Bacteroides* and *Spirochaetae* were dominant in the TB group compared to the control group, the frequency of *Burkholderia, Fusoberierieria Furvolaeri, Burkhold* and *Pasteurellaceae* increased significantly in the normal group compared to tuberculosis patients (Figure 1).

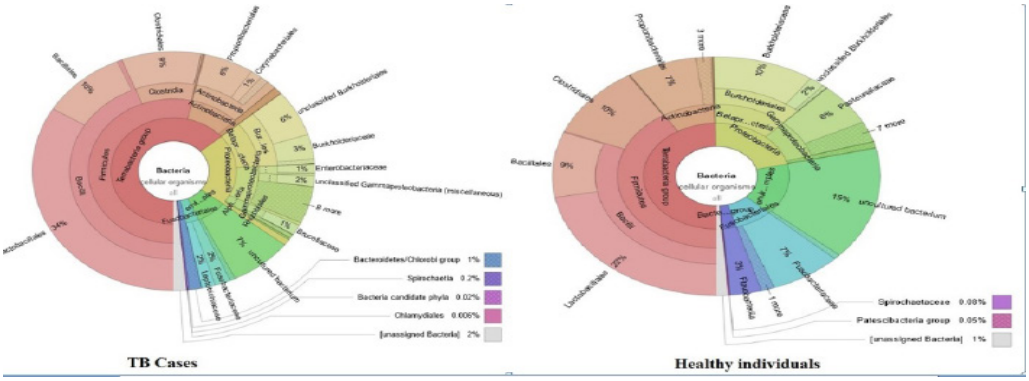

Figure 1. Distribution of lower respiratory tract microbiota in TB cases vs. healthy individuals.

Discussion

In this study, five bacterial phyla that were most frequent in BAL samples included *Firmicutes, Proteobacteria, Actinobacteria, Fusobacteria*, and *Bcteroides*, which are also found in other anatomical areas of the body such as skin, gastrointestinal, oral cavity, and upper respiratory tracts (16). There are several studies on the role of these bacterial phyla in chronic obstructive pulmonary disease, asthma, cystic fibrosis, and other respiratory disorders (17,18). However, according to our study, microbial diversity was similar between TB patients and healthy individuals. Lam et al. (2016) also showed that the microflora in sputum is similar to healthy individuals and patients with tuberculosis (19).

Among the major identified genera, *Streptococcus, Propionibacterium, Staphylococcus, Neiseria, Fusobacterium*, and *Leptotrichia* significantly increased in tuberculosis patients, which appear to play a central role in the progression to active-TB. According to the review of the literatures, all of these bacterial genera have been isolated from respiratory disorders and cause pneumonia. Increased anaerobic genera also restrain the immune system by increasing succinate and its anaerobic metabolism (20, 21). Zhou. (2015) also showed that the number of anaerobic bacteria, and particularly *Porphyromonas*, was inside within granuloma lesion than other regions (22). Many similar studies have also identified members of *Enterobacteriaceae, Violonella, Leptotrichia* from among microbiota of sputum in tuberculosis patients. *Ruminococcus, Thiomonas*, and *Leptotrichia* appear to suppress the immune response by producing their own metabolites, colonizing anaerobic bacteria such as *Fusobacterium, Propionibacterium*, and *Porphyromonas*. Also, members of *Enterobacteriaceae* cause pneumonia and their high incidence in tuberculosis patients can be considered as a risk factor for tuberculosis susceptibility (22-25). In the present study, the microbial diversity and abundance of microbial community in BAL samples were similar to the results of other metagenomics studies on sputum specimens of tuberculosis patients, indicating the validity and reliability of the results (23, 24, 26). The results of our study supported the previous studies, although there were differences in the abundance of bacterial genera in some cases, which may have been related to the evaluated samples (BAL and sputum). However, the interpretation of the relationship and collaborative function of bacteria in the lower respiratory tract microflora was very complex in tuberculosis patients and requires further studies.

Conclusion

We showed that microbial diversity of lower respiratory tract microbiota in tuberculosis patients is similar to that of healthy individuals, although the abundance of several specific genera was significantly different between the two groups. Variations in the frequency of microbial community of lower respiratory tracts microflora appear to be a risk factor for increased risk of tuberculosis.

Conflict of Interest

The authors declare no conflict of interest.

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